REVIEW OF THE 2001 CHASE ENCIRCLEMENT STRESS STUDIES ON DOLPHINS TARGETTED IN EASTERN TROPICAL PACIFIC OCEAN PURSE SEINE OPERATIONS

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EXECUTIVE SUMMARY

An ensemble of studies was conducted on pantropical spotted dolphins (*Stenella attenuata*) and eastern spinner dolphins (*Stenella longirostris*) to determine the physiological effects of tuna fisheries on these species in the Eastern Tropical Pacific Ocean (ETP). This series of studies addressed complementary aspects of the stress response. It has not provided definite evidence to support that the chase and encirclement associated with the fishery have negative effects on the dolphin populations.

Previous work and the observations reported in CIE-SO6 have documented passive dolphin behavior that is consistent with the clinical signs of exertional myopathy (EM), a disease first described in free-ranging ungulates forced to strenuous exercise by chase carried out with motor vehicles. This disease has been reported in virtually all vertebrates, including pinnipeds and cetaceans, since earlier work on free-ranging ungulates.

Previous post mortem examination on a small number of ETP dolphins has failed to show morphological evidence of this disease. The findings reported in CIE-SO2 and CIE-S11 suggest that this disease affect some dolphins. They warrant the reexamination of the results from the hematological parameters study (CIE-SO2) and the examination of skeletal muscles of a large number of dolphins because the evidence required for a definite diagnosis of EM consists of morphological manifestations of damage to skeletal muscles. In addition, the availability of baseline data in captive *S. attenuata and S. longirostris* would definitely consolidate the interpretation of the hematological parameter study (CIE-SO2) regarding the diagnostic of EM.

Dolphins severely affected by EM (or by any other disease) are more likely to be killed and destroyed by predators, and thus are likely to be underrepresented in samples of live animals. Thus, any future study addressing the health status of ETP dolphins should target a larger number of dolphins, at least several hundreds, because severely diseased dolphins are likely to be rapidly destroyed by predators. In this regard, the dolphins currently killed in the tuna fisheries constitute a large and relatively cheap source of material that should be urgently exploited especially with regards with the examination of skeletal muscles. Post mortem examination of these carcasses would be an inexpensive way to detect pathological changes, such as those induced by EM, in a large number of animals.

The thermal studies (CIE-SO4) did not show evidence of hyperthermia, an important clinical sign of EM. This suggests that heat-dissipating mechanisms are highly efficient in ETP dolphins, and that dolphins are efficiently protected against hyperthermia. In CIE-S14, the authors suggest that dolphins may have been trained to avoid tuna fishing operations. In the same line of thought, ETP dolphins may have been massively and negatively selected over 50 years by tuna fisheries for resistance to hyperthermia and possibly to EM.

The Skin Molecular Stress Response studies (CIE-SO7, CIE-SO8) addressed an interesting and promising concept. The interpretation of results however was hampered by several troublesome, technical problems. The studies that evaluated the immune functions (CIE-SO3, CIE-S10) did not reveal any immune dysfunction with the important exception of an imbalance between B and T cells. This type of imbalance has been

related to extensive exercise in other species. This abnormality should be further investigated.

CIE-S12 and CIE S13 bring mathematical and behavioral observations that strongly suggest a negative impact of the chase on the mother-calf pair. According to other studies on free-ranging cetaceans, an increasing proportion of calves is expected over the years in a recovering population. On the contrary, the authors of CIE-SO15 report a significant decline in proportion calves over a ten-year period.

Considered together, these studies support that tuna fisheries impose a high stress upon ETP dolphins and possibly disrupt the mother-calf pairs. In addition, the results suggest that exertional myopathy triggered by the chase and encirclement may affect an undetermined percentage of animals.

RECOMMENDATIONS

- **To determine the baseline biochemical parameters of** *S. attenuata* **and** *S longirostris.*
  It is important to determine the baseline levels of these parameters in normal, resting domesticated or semi-domesticated *S. attenuata* and *S longirostris.* This would allow the interpretation of the biochemical parameters measured during the CHESS study, particularly with regards to muscle enzymes and HCO_3 levels.

- **To test the statistical relationship between alterations in CK, AST, LDH, blood pH (acidosis), HCO_3, anion gap, lactic acid and K^+ for each animal with high CK values.**

- **To carry out post-mortem examinations of a (representative) large number of dolphins killed in seine nets or during chase with an emphasis on the examination of skeletal muscles.** See Wobeser (1994) for an extensive discussion of the number of animals to be examined.

- **To determine whether tuna fishing causes post-release mortality in ETP dolphins**
  To achieve this goal, it is necessary to tag a large number of ETP dolphins captured in a simulated fishery with radio transmitters. Tagged animals will be tracked for three weeks to evaluate mortality and if possible, carcasses will be recovered and examined.

- **Reproductive studies**
  The carcasses should be examined with regards to reproductive success/physiology.

BACKGROUND

Eastern Tropical Pacific (ETP) dolphin populations have sustained heavy losses due to the tuna fishing industry. A particular method used by the industry since the late 1940s is based on the spatial aggregation of dolphins and tuna, and consists of entrapping both dolphins and tuna with a net, the purse seine (National Research Council 1992).

From 1959 to 1972, this method, termed "dolphin fishing", caused the death of at least 100,000 dolphins a year with a cumulative loss of 4.9 million dolphins from 1959 to 1972 (National Research Council 1992; Wade 1995 in Curry and Edwards 1998). In 1972, the Marine Mammal Protection Act, along with the continuing improvement of techniques and equipment and the increased attention of fisherman, all decreased the total observed mortality to 25,000 dolphins in 1991 (National Research Council 1992).

In 1995, the Declaration of Panama was signed between the USA and eleven other tuna fishing countries to decrease dolphin mortality to a total of 5,000 a year with the ultimate goal to eliminate it. In 1996, the total observed mortality was 2,547 dolphins (Lennert and Hall 1997 in Curry and Edwards 1998).

In 1997, the Marine Mammal Protection Act was amended by the International Dolphin Conservation Program (IDCPA) to implement the Declaration of Panama. This allowed the importation of yellowfin tuna in the USA from other fishing countries under the label "dolphin safe" if dolphin injury or mortality is not observed during fishing operations. Because of the concern that purse seine "dolphin fishing" may still be detrimental to the dolphin stock, the IDCPA required that research be conducted by the National Marine Fisheries Services (NMFS) to determine if the seine fishing method has negative effects on the ETP dolphins (Curry and Edwards 1998).

From August to October 2001, a 2-month research cruise was carried out in the Eastern Tropical Pacific Ocean to determine whether repeated chase and encirclement have a negative impact by inducing stress on pantropical spotted dolphins (*Stenella attenuata*) and eastern spinner dolphins (*Stenella longirostris*).

The present work is a review of the studies conducted during that cruise. It was requested to the author by the "University of Miami Independent System for Peer Reviews" under a consulting agreement.

DESCRIPTION OF REVIEW ACTIVITIES

Fourteen scientific reports from the CHESS research cruise and from demographic studies of ETP dolphin were sent to this and three other reviewers, and these were examined two weeks before the three-day meeting, held at the NMFS La Jolla Laboratory (Feb. 4 to Feb. 6 2002).

The La Jolla meeting consisted of oral presentations given by the authors of each study. Each presentation was followed by questions and comments from the four reviewers.

The literature pertaining to diseases and physiology of cetaceans, particularly with regards to stress and capture, was examined. The scope of the literature review was extended to diseases and stress affecting free-ranging ungulates and more generally free-ranging mammals.

The summary of findings, individual review of each paper, conclusions, recommendations and references follow.

SUMMARY OF FINDINGS

At the onset of this review, the investigators have to be commended for their commitment to elucidate the potential decline of ETP dolphins. This study required spending two months at sea in sometimes-difficult weather in a remote region of the Pacific.

Based on the documentation that was made available to this reviewer, it is possible but not certain that the ETP populations do not recover from the heavy losses due to tuna fisheries. Stress has been suspected to explain this possible failure to recover. To this reviewer's knowledge, stress alone has never been documented to cause the decrease or the failure to recover of a population of wild or domestic animals. This does not rule out the possibility that stress impacts ETP dolphins but it makes that hypothesis less likely especially when there are much better documented and more frequent causes.

It is true that at the individual animal level, however, stress alone has been shown to cause severe damage, but mostly in experimental models. Fatal heart muscle necrosis is seen in laboratory animals submitted to highly aggressive challenges (see the extensive review by Van Vleet and Ferrans 1986). There is a single apparent exception. The so-called "porcine stress syndrome", also termed malignant hyperthermia, is a genetic disease secondary to mutations in the ryanodine receptors gene (Missiaen et al 2000). Acute stress such as stress caused by transportation, high temperature, high humidity, running, fighting, or mating can kill the pigs with the mutations because cytosolic calcium levels increase suddenly in cardiac and skeletal muscle fibers following catecholamine release. The myocardium of affected pigs show hyaline necrosis or contraction band necrosis (Van Vleet and Ferrans 1986).

Gastric ulcers that perforate or cause hemorrhages are often the cause of death of cows and pigs that are under physiological stress. Stress was believed to play a major role in the development of these lesions. However, as in people, it is increasingly believed that *Helicobacter* infections play an important role in the etiology of these lesions.

Acute stress has been suspected 1) to contribute to the etiology of a fatal disease of wild mammals termed exertional myopathy (EM), and 2) to increase the incidence of infectious diseases in domestic/laboratory animals and in humans (Peterson et al 1991). By contrast, increased mortality, loss of habitat, and decreased reproductive success have been well-documented, major causes of the reduction of wild mammal populations (Fujiwara and Caswell 2002; Thompson and Hamer 2000; Pimm and Raven 2000). Thus, it is important to determine whether tuna fishing induces deaths in ETP dolphin. There is ample evidence that the mortality of ETP dolphins directly caused by the tuna fisheries has been dramatically decreased. Thus, if mortality occurs, it must be after animals are released from the nets.

In addition, over the last two decades, there have been an increased number of instances where diseases have been suspected to play an important role in the decrease or failure of recovery in terrestrial populations (Carpenter et al 1998), as well as marine mammals (Lipscomb et al 1994; van de Bildt et al 1999). These diseases have been detected by examining carcasses stranded on the shoreline. It is doubtful that such mortality would have been detected in pelagic species such as *S. attenuata and longirostris*.

There are also several reasons why the physiological quantification of stress or of its impact on a free-ranging cetacean species are unsettling to this reviewer, especially during the course of a single capture-recapture event.

Firstly, not only is the current definition of stress vague but in addition, every researcher in the field has a different definition of the concept (In the 1980st his reviewer attended a presentation in Montreal, Canada, of the then retired Hans Selye - the researcher who originally invented both the "stress" word and concept. In that presentation, Dr Selye described, as in his original paper, a well characterized syndrome defined mainly by a trilogy of lesions: Gastric ulcers, adrenal hemorrhages and thymic atrophy. This precision may have been due to the aggressive and prolonged nature of the experimental challenges that he used (Selye 1937)).

Secondly, stress is notoriously difficult to measure in humans and domestic animals despite the extensive knowledge of normal physiological and blood parameters in these species (Fowler 1995). Thus, it is difficult to envision how stress could be measured during the course of a single capture-recapture event in free ranging ETP dolphins, of which the normal biological and physiological data were basically unknown. Note that the problems inherent in measuring stress also explain why the precise contribution of stress to the etiology of EM is still under debate.

Odontocetes are taxonomically related to ungulates. Dolphins and ungulates have a common ancestor, *Pakicetus*, a small ruminant that lived 50 millions years ago (Bajpai and Gingerich 1998). This evolutionary relationship may explain why infectious agents such as Morbilliviruses, that resemble those that affect ungulates, have caused severe epizootics in whale populations worldwide over the last two decades. Morbilliviruses are genetically similar to those causing "Peste des petits ruminants", a deadly disease of ungulates.

The ontogenetic relations between ungulates and cetaceans extend to exertional myopathy (EM), a metabolic disease first recognized in Africa in free-ranging ungulates chased by air and ground motor vehicles. The disease often leads to delayed death of all free-ranging ungulates chased in the same condition, when they are forced to strenuous exercise and submitted to extreme stress (Williams and Thorne 1996).

EM has been variously termed capture myopathy, exertional rhabdomyolysis, overstraining diseases, stress myopathy. EM is manifested by pathological changes characterized by damages to the skeletal and cardiac muscle, the elevation of serum levels of enzymes normally present in intact muscle fibers, metabolic acidosis and hyperthermia. In people and some ungulate species suffering EM, damaged muscle fibers release myoglobin that is eliminated in the urine (myoglobinuria). In these less frequent cases, myoglobinuria may trigger renal damage, particularly in proximal tubules (Gitin 1974; Williams and Thorne 1996).

Over the last two decades, EM has also been described in birds, canids, marsupials, and even fish. It is now thought that all animals may suffer the disease when they are submitted to strenuous exercise accompanied by excitation (Williams and Thorne 1996).

Others have previously reported EM in cetaceans and pinnipeds (Simpson and Cornell 1983), and a suspected case of EM has been described in a transported Pacific bottle-nosed dolphin (*Tursiops gilli*) (Colgrove 1978).

Considering the universal presence of EM in animals, because EM has been described in pinnipeds and cetaceans and because of the ontogenetic relationships between wild ungulates and dolphins, it is very likely that a certain percentage of spotted dolphins chased during tuna fisheries operations also suffer EM.

Importantly, in 1979, EM was already suspected in the ETP dolphin populations. Sixty five carcasses killed during the fishing operations were dissected and carefully examined, including skeletal muscles. After careful gross and microscopic examination of the skeletal muscles, no evidence for EM was found (Cowan and Walker 1979).

For this reviewer, there are several possible explanations for the absence of EM lesions in these earlier studies. Free-ranging ungulates with severe EM are predisposed to predation and accident (Williams and Thorne 1996). Consequently, most explanations listed below are based on the assumption that ETP dolphins with the severe form of EM cannot survive in the wild because they become easy prey to predators such as sharks:

1) **An insufficient number of animals may have been sampled.** The number of animals that must be examined in order to detect a disease with a given prevalence in a population of free-ranging animals has been discussed in minute details elsewhere (Wobeser 1994). For instance, with an animal population size over 1,000,000 animals, and a suspected disease prevalence of 0.5 %, 600 animals should be examined, **that is, about 10 times more than the number of ETP dolphins examined** in the current study (CSE-S11) and in 1979 (Cowan and Walker 1979). A 2% prevalence requires that 150 animals be examined, about three times more than the number of ETP dolphins examined here and in 1979. A 10% prevalence requires that 30 animals be examined (Wobeser 1994). Note again that if the animals most severely affected by EM do not survive in the wild after their release, the percentage of live animals suffering severe EM is more likely to be lower. An additional consequence is that survivors have less severe lesions, and in turn these lesions are more difficult to detect upon post mortem examination.
2) **The "non response" problem (biased selection) may affect the random sampling method that was used for sampling.** The "non response" problem is the equivalent of people who do not respond to questionnaires in epidemiological studies. In wildlife epidemiological studies for instance, older, more experienced animals may avoid capture, which results in a sample composed of younger animals showing an entirely different pattern of disease (if they show any disease) (Wobeser 1994).
3) **EM may affect muscles other than those sampled.**
4) **Small herds have been sampled** in the CHESS program compared to the size of herds pursued in the usual operations (CIE-SO1). Because chasing large herds of ungulates predisposes to EM, the chase of small dolphin schools may lower the prevalence of EM in captured dolphins (Kock et al 1987a).
5) **Dolphins may be progressively trained to avoid the capture (and thus EM) associated with tuna fishing**. Mesnick et al (CIE-S14) provide a fascinating review supporting that dolphins avoid and escape tuna fishing in the most extensive fishing areas. Consequently, as some (or most ?) dolphins get trained over the years, they would avoid EM, which would lower or suppress the incidence of the disease in the population.
6) **All the above explanations may coexist.**

7) **No ETP dolphins suffer EM**. That would make these animals unique among free-ranging mammals and even vertebrates. This uniqueness is not impossible (but is unlikely). This uniqueness is not impossible because hyperthermia, which is believed to play an important role in the development of EM, is probably rare in healthy marine mammals kept in water because water conducts heat more easily than air, and the specific heat of water is high (see also CIE-SO4). However, EM has been previously diagnosed in cetaceans and pinnipeds. Thus, the possibility that ETP dolphins do not suffer EM is remote.

Veterinarians and biologists involved in the capture of wildlife are well aware that exertional myopathy should be one of the most important considerations when planning and executing operations that require handling of wild animals, and every effort should be made to avoid inducing disease (Williams and Thorne 1996).

The prevalence of capture myopathy in free-ranging ungulates varies from less than 4 % in bighorn sheep (Kock et al 1987a) to 36 % in blue wildebeest (Bartsch et al 1977) chased and/or captured particularly by mechanical means or restrained. We expect that the prevalence of spotted dolphins affected by EM is within that range (but the *apparent* prevalence would be lower because predators will most likely attack and destroy these dolphins).

The most frequent reports of EM are from animals that have been forced to a strenuous exercise, even of short duration, because of trapping, pursuit, capture, restraint and transport, even in animals that have naturally evolved for long sustained running either for evasion or predation (Williams and Thorne 1996).

The pathogenesis of EM may be related to shock triggered by stimulation of the sympathetic system (Spraker 1980, 1982, 1993). According to others, extreme effort is more important. Extensive effort triggers hyperthermia and metabolic acidosis (Chalmers and Barrett 1982). One minute of extensive exercise can decrease blood pH to 6.8 (Myers 1995). Metabolic acidosis decreases cardiac output, which results in decreased systemic blood pressure, a major feature of shock. Metabolic acidosis is more pronounced in animals forced to run at high speed over short distances than in animals forced to run at a lower speed over a longer distance (Harthoorn and Van Der Walt 1974).

The average travel speed of ETP dolphins is 4.9 knots (KN) and 3.7 KN for daytime and night time, respectively (Chivers and Scott, CIE-SO5). During purse -seine operations, the average chase speeds of two dolphins were 3.4 and 5.7 kn. The range was not provided however (maximum and minimum speed). These averages could mask a series of sprints at 7 KN, and then would not reflect the actual physiological demands put on these animals. In 1992 and 1993, the average speeds were higher (5.3 KN) (in Chivers and Scott CIE-SO5). Again it would be important to reinterpret the data using the upper speed values, not the average speed.

There are striking similarities between the factors causing EM in ungulates and the methods used to chase ETP dolphins. Helicopters and speedboats are used to chase ETP dolphins. The chase of ungulates with mechanical vehicles, including helicopters, has long been a well-known factor causing EM. For instance, Kock et al (1987a) compared different capture methods regarding their potential to cause EM in pursued free-ranging bighorn sheep. They recommended: "utilizing the most experienced and skillful helicopter pilot and net gunner and an appropriately powered helicopter"… "The helicopter herding of bighorn sheep must be conducted slowly until the last minute, and prolonged pursuit must be avoided (< 10 minutes). Herding of large groups (> 10 to 12 sheep)" of sheep should be avoided (Kock et al 1987a).

In comparison, the average duration of the spotted dolphin chase is 20 to 40 minutes but can sometimes take more than 2 hours, and the average herd of spotted dolphin is composed of 400 animals (Curry and Edwards 1998) (National Research Council 1992). Thus the conditions under which ETP dolphins are chased seem highly favorable for inducing EM. Note that the CHESS study was conducted with smaller dolphin herds (average of 59 dolphins) than those chased in commercial operations CIE-S01), which could contribute to lessen the prevalence and severity of EM.

Ungulates with EM die within several hours after the chase, most affected animals die 2 to 4 days after the chase, and a few die 1 to 4 weeks after (Van Vleet and Ferrans 1986). Early signs other than hyperthermia "include depression, lack of response to the environment and recumbency (Harthoorn 1973). …Free-ranging animals in this condition are predisposed to predation and accident" (Williams and Thorne 1996). These early signs are strikingly similar to the behavior of some spotted dolphins that have been chased and encircled.

Netted dolphins may be grouped in two classes, rafting and active animals, according to their level of activity (National Research Council 1992). "Rafting animals hang quietly. Rafts include passive columns of animals as deep as 20 meters below the surface that may from time to time rise to the surface" (Pryor and Kang 1980).

The above observations strongly suggest that netted "rafting" dolphins and/or other netted dolphins showing a passive behavior suffer in fact acute exertional myopathy. This hypothesis predicts that the hematological biochemical parameters typical of EM will be most apparent in "rafting" animals (See CIE-S02). If this hypothesis is confirmed, "rafting" animals should not be subjected to additional disturbance after encirclement. On the contrary, treatment for EM might be envisaged for these animals.

Animal affected by EM may die suddenly or develop signs hours, days, or weeks after the chase (Basson and Hofmeyr 1973). Warm weather probably renders animals susceptible to EM. Ungulates species differ in their susceptibility, and these differences may be due to the different methods of capture and transport that are used (Basson and Hofmeyr 1973; Chalmers and Barrett 1977; Williams and Thorne 1996).

Skeletal muscles lesions are seen in most species while lesions to the cardiac muscle are less frequent. In some species, however, cardiac lesions have been reported without skeletal muscle lesions (Van Vleet and Ferrans 1986; Williams and Thorne 1996). Skeletal muscle lesions are usually bilateral but not necessarily symmetrical (Bartsch et al 1977).

The nature of the muscle lesions varies with time. From 10 to 24 hours, the lesion in the damaged muscle is dark red, dry and well demarcated. Microscopically, affected muscle fibers show loss of striations, waviness, and hyalinization. The sarcoplasm is deeply eosinophilic and homogeneous, or finely granular. The sarcoplasm can contain eosinophilic globules or vacuoles. Some fibers are generally fragmented with lysis, hemorrhages and occasional mineralization. Frequently, nuclei are pyknotic, displaced centrally or are absent. (Bartsch et al 1977; Williams and Thorne 1996). Similar lesions have been described in the heart (Van Vleet and Ferrans 1986).

After 3 to 4 days, the lesion has a lighter color and its consistency is soft or gelatinous. Microscopically, muscle necrosis is accompanied by multifocal mineralization, infiltration of necrotic foci by neutrophils and macrophages, and there is evidence of muscle fiber regeneration (basophilic fibers, proliferation of large sarcolemmal nuclei) (Bartsch et al 1977; Williams and Thorne 1996).

After one or more weeks, the muscle becomes white and hard to cut, which reflects scarring characterized by fibrosis.

CIE-SO1. Chase encirclement stress studies on dolphins involved in Eastern Tropical Pacific Ocean Purse Seine Operations during 2001.

KA Forney, DJ St Aubin, SJ Chivers.

General comments

This is an excellent review of the sum of work carried out in the CHESS study. This reviewer entirely agrees with the major conclusions of this synthesis, that is that CHESS study has not provided definite evidence to support that chase and encirclement leads to negative effects on the ETP population.

Specific comments

On p 15, several reasons are provided to explain the low number of lactating females in the sample. Although the sample may be biased, one obvious reason is that there could be low numbers of lactating females in the population.

I agree that the high levels of catecholamines are consistent with the *acute* hart lesions found in dolphins killed during CHESS operations. On p. 26, the authors mention lesions in skeletal muscles. Those have not been described in CSE-SO11.

On p. 17, in the first paragraph, it is not clear to this reviewer why the authors use the modifier "benign" in "benign myopathy". At this stage the reviewer cannot agree that the muscle enzyme levels are consistent with subtle muscle lesions, because pooling of the data may have obscured a potential diagnosis of EM. In order to diagnose EM (or any other pathological condition), blood parameters should be evaluated individually for each animal with high CK, not pooled. Specifically, the blood parameters should be considered individually to determine if a given animal with upper values for CK also shows an elevation of the other seven parameters related with EM (i.e. low HCO_3, high lactate, anion gap, CK, AST, high K^+). Moreover, the results of the skeletal muscle examination are not reported in CIE-S11.

The concept of using the skin as a window to quantify molecular responses to stress is both exciting and promising (CIE-SO8). Many technical hurdles remain to be surmounted however.

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CIE-S02. Hematological and serum chemical constituents in Eastern Spotted Dolphins (S. attenuata) following chase and encirclement

D St Aubin.

General comments

Some of the blood parameters measured in this study suggest that exertional myopathy affects some dolphins.

Specific comments

EM is characterized by eight altered blood parameters which are by order of decreasing specificity: increased CK, AST, LDH, acidosis, decreased HCO_3, increased anion gap, increased lactic acid and increased K^+ (Williams and Thorne 1996).

Some of them, such as pH, HCO_3 and anion gap, are interrelated, and some of them, not all, are expected to be altered in any given affected animal. Elevated "muscle" enzymes (CK, AST and LDH) characterize EM because enzymes leak from damaged muscle fibers. Of these, CK is the most specific indicator of muscle damage. Elevation of CK levels in domestic animals and people is a sign of active or very recent muscle degeneration or muscle necrosis (Cardinet 1989). Serum CK levels of red deer with severe muscle damage are markedly elevated (35 - 47,500 U/L) (Wilson and Pauli 1983). The CK values in the CHESS ETP dolphins reached 560 U/L, which is significantly higher than in other cetaceans, that is, between 49 and 200 U/L (Colgrove 1978; reviewed in Bossart et al 2001).

AST is not as specific for muscle damage as CK since AST is also present in brain, liver and red blood cells. Its elevation however may strongly suggest the presence of muscle damage in the absence of lesions in the other tissues where it is normally present. AST serum levels take longer to increase following muscle damage and the elevation persists longer than that of CK. AST values in the CHESS ETP dolphins reached 520 U/L, which is significantly higher than in other cetaceans, that is, generally below 100 U/L.

LDH levels were also elevated in ETP dolphins, reaching an upper value of 1308 U/L when values in other cetaceans are normally below 600 U/L (Colgrove 1978; reviewed in Bossart et al 2001).

Dr. St Aubin noted that mean levels of CK, AST, and LDH were all elevated compared to reported values for several species. To this reviewer and to Dr. St Aubin, this observation strongly suggests that the diagnostic of EM should be considered. Dr. St Aubin also observes about the high levels of these enzymes "but (these levels) are similar to those reported for net impounded harbor porpoises". For this reviewer, there is no opposition between the high muscle enzymes levels found in ETP dolphins and impounded porpoises on one hand and the diagnostic of EM in ETP dolphins on the other hand. Porpoises caught in nets for days are very likely to have severe muscle damages, which elevate serum muscle enzyme levels (Baker and Martin 1992).

The "muscle enzymes" (ALT, CK and AST) values collected in 1977-1978 in ETP dolphins are lower than the values collected in the current study (Table 3). The author comments that these discrepancies are probably due to the different methods used.

I suggest that instead, or in addition, this discrepancy may be due to a different method used for capturing the animals in 1977-78.

In people and animals, metabolic acidosis (low blood pH) can be caused by the loss of HCO_3 or by the accumulation of organic acids such as lactic acid. A major alteration in EM is metabolic acidosis caused by the accumulation of lactic acid. Muscle contraction requires ATP production, which in turn increases the need for oxygen. If the needs for oxygen increase to a point that oxygen cannot be delivered fast enough to the working muscles, anaerobic glycolysis develops, which generates lactic acid (Read et al 2000). Lactic acid accumulation leads to metabolic (lactic) acidosis, hypotension, shock and death.

Zebras forced to run illustrate a dramatic example. Three zebra chased for 1, 2 and 5 kilometers developed metabolic acidosis with a blood pH of 6.5 after running only 1 to 5 km. Half an hour later, one animal died. The blood pH of the other two continued decreasing to 6.45 and both died within 12 hours of capture (Harthoorn and van Der Walt 1974).

The accumulation of lactic acid (and other organic acids) in blood causes the elevation of the so-called "anion gap" (Duncan et al 1994). Because an important feature of EM is the accumulation of lactic acid in blood, EM is also accompanied by an elevated anion gap.

Metabolic acidosis is considered moderate in most domestic species when HCO_3 serum levels are 15-20 mEq/L, and 12-17 mEq/L in dog and cats (Duncan et al 1994). In most domestic species, metabolic acidosis is considered severe when HCO_3 levels are below 15 mEq/L and below 12 mEq/L in dogs and cats. In comparison, the range reported in the ETP CHESS dolphins includes values that are clearly low (10-29mEq/L).

In domestic animals, the anion gap is normal between 12 and 16mEq/L. In comparison the anion gap values reported in the ETP CHESS dolphins include values that are clearly elevated (up to 43mEq/L; average 17.3mEq/L).

In people and animals, EM is rarely accompanied by renal damage, and consequently there is no increase in BUN and creatinine (Gitin 1974; Williams and Thorne 1996). Note that free-ranging ungulates affected by capture myopathy have normal Cl^- levels, like the ETP CHESS dolphins.

Diagnostic

EM affects between 4% to 36% of free-ranging wild ungulates of the same species submitted to strenuous exercise during chase. Pooling the clinical data of blood biochemical parameters does not allow for the detection of a disease affecting a relatively small proportion of animals or humans within a population. Thus, in order to diagnose EM within a population of chased animals, either ungulates or dolphins, the set of biochemical data relating to muscles must be examined on an individual basis.

It is impossible to know if a given dolphin shows the set of biochemical alterations typical of EM; that is, if a given animal is affected concurrently by metabolic acidosis (low HCO_3), high serum levels of muscle enzymes, and hyperkalemia. By analogy, it would be impossible to detect cases of diabetes mellitus in a human or animal population if all values of blood glucose levels of all individuals were pooled and averaged (unless the proportion of affected people or animals was very high and/or the values really high). The same reasoning holds for a variety of (if not all) medical conditions of which the diagnostic relies on quantitative clinical tests.

Consequently, this reviewer suggests testing the statistical relationships between alterations in CK, AST, LDH, HCO_3, anion gap, lactic acid and K^+ for each animal with high CK values. It would be desirable also to determine the proportion of animals affected in that manner.

Ten CHESS dolphins were recaptured and of these, only two were sampled twice (as far as is known) (Tables 8 and 9). These animals did not show elevated serum muscle enzymes compared to the levels measured in the course of the first capture. The absence of muscle enzymes elevation upon second capture is consistent with EM: in free-ranging cervids submitted to weekly capture, the elevation of muscle enzyme is most apparent during the first capture.

The sampling design and the scarce data (two animals sampled twice) create some problems for interpretation: 1) The data from the first capture cannot be considered baseline data since they come from animals heavily exerted and/or stressed. Thus the values from these animals are not from "normal" animals. 2) As explained by Dr St Aubin during his presentation, it was impossible to determine whether any given animal had been captured and sampled before. Thus any given captured animal could have been captured an indefinite number of times, thus altering its blood parameters. According to CIE-SO1, the probability of recapturing animals by chance is remote because of the low "fishing effort" made by the research cruise. 3) The relatively low frequency of EM (4-36%) in ungulates makes it unlikely to detect affected dolphins if a small number of dolphins is examined.

Finally, the author observed that spotted dolphins showed an exaggerated response to stress based on the observed high and blood levels of catecholamine, ACTH and cortisol, glycemia and the high hematocrit. Stress is believed to play a role in EM. Accordingly, Dr St Aubin concluded that elevated catecholamine levels highlight the risk for benign capture myopathy. Why the "benign" modifier?

Considered together, the above observations support the diagnostic of EM in some ETP dolphins. The evidence missing for a definite diagnosis are samples of skeletal muscles demonstrating muscle damage concomitant with increased muscle enzymes, anion gap, increased HCO_3 and K^+. The availability of baseline data for serum muscle enzyme levels in captive *S. attenuata* and *S. longirostris* would definitely consolidate the interpretation of the muscle enzyme data.

CIE-S03. Investigation of the effects of repeated chase and Encirclement on the immune system of Dolphins (S. attenuata and longirostris) in the Eastern Tropical Pacific

T Romano, M Keogh, K Danil

General comments

Stress is known to alter the composition of the various classes of lymphocytes that circulate or reside in lymphoid and non-lymphoid tissues. Thus it was logical to evaluate whether such changes occur in ETP dolphins subject to the chase stress.

Specific comments

The authors determined the variations occurring in the percentages of the major classes of lymphocytes in ETP dolphins between a first and a second capture. It was found that the percentage of B cells decreased and the percentage of T cell increased, a change similar to that seen in human marathoners and restrained animals (Dhabhar et al 1995; Gmunder et al 1988). The comet assay, used to assess DNA damage, did not detect any DNA damage in lymphocytes.

The lymphocyte count (lymphopenia) was consistent with changes seen in stress when dolphins captured once were compared with dolphins captured twice, and were matched for gender, length, and girth (Table 3). The authors mention that data from dolphins captured once are not valid baseline data, since different dolphins composed the two groups. In addition, the parameters measured in dolphins submitted once to the chase do not reflect the basal parameters of *S. attenuata* and *longirostris* at rest.

CIE-SO4. Measuring temperatures and heat flux from dolphins in the Eastern Tropical Pacific: is thermal stress associated with chase and capture in the ETP-Tuna Purse Seine Fishery ?

D Ann Pabst, WA McLellan, EM Meagher, AJ Westgate.

General comments.

Heavy exercise in mammals increases body temperature because muscular contraction results in increased catabolism, which generates heat. Hyperthermia leads to brain, liver and kidney damage in mammals. Hyperthermia in pregnant animals can lead to fetal damage of the central nervous system, mostly if it occurs in early pregnancy.

Hyperthermia has been reported as a major factor contributing to the etiology of EM (Chalmers and Barrett 1982). Captured bighorn sheep (Kock et al 1987b), immobilized white-tailed deer (Seal and Bush 1987), and zebra (Harthoorn and Young 1974) were submitted to simulated capture exertion. The animals with temperatures respectively greater than 42^{o}C, 40^{o}C and 41.5^{o}C subsequently died. Thus, this part of the CHESS study was important and necessary.

Specific comments.

Three different approaches were used to evaluate heat production and possible overheating. The first technique, which measures deep core body temperature with a rectal probe, showed that the longer females were chased, the lower their body temperature was. This result could have been influenced by the position of the probe however because in dolphins the counter current heat exchange system is close to the colon. (The authors have described elsewhere this heat dissipating system, which protects the reproductive system (and the potential foetus) from overheating). The second technique, infrared thermography, measures skin temperature. It captured data that, although highly variable, indicated that skin temperature increased proportionally to chase duration. Thus, dolphins shunt blood to their periphery to successfully get rid of heat. The third technique, thermal data logger deployments, was used to quantify heat flux in two dolphins. The values were within the range observed in other dolphins, both wild and captive.

To summarize, the ETP CHESS dolphins submitted to heavy exercise seem to dissipate heat efficiently, without life threatening increases in body temperatures after or during chase. For this reviewer, potential explanations include that ETP dolphins may have easy access to cold water and/or that ETP dolphins have efficient physiological mechanisms to dissipate heat. A third explanation is that over five decades, the extensive tuna fisheries have either selected or trained at least some ETP dolphin populations for hyperthermia resistance. Note that is has been shown that free-ranging ungulates become resistant to EM through physical training (Harthoorn 1980).

Purse seine nets are 600 feet deep (or about 180 meters) (National Research Council 1992). The thermocline was about 25 to 35 m deep at the time and location of the CHESS study whereas dolphin's dives "rarely exceeded 5 m" (Chivers and Scott. CIE-SO5). Could the thermocline be disturbed, disrupted and/or shallower within the net enclosure? Shallow cold water would explain that the entrapped dolphins do not need to dive deeply to "cool off " in the net enclosure. Could the huge purse seine apparatus and the ample ship maneuvers hauling the large net appendage make the thermocline shallower, or could they disrupt it, mixing warm water surface with cold, subthermoclinal water?

Alternatively, when dolphins rarely exceeded 5 meters within the net enclosure, perhaps they sojourned in the subthermocline cold water long enough and/or frequently enough to cool off. (For anybody who practices scuba or snorkel diving, reaching the thermocline is a brutal experience, with or without a wet suit).

Figures 29 and 31 show the dramatic drop in heat flux of two dolphins released from the net while their body temperature remains constant. After this post-release initial drop in heat flux, the pattern is characterized by sharp oscillations of which the frequency is about 20 seconds. The initial dramatic drop and the subsequent decreases may represent deeper dives in the colder subthermocline water.

Perhaps dissipating heat does not even require dolphins to reach the thermocline. These animals have highly efficient heat dissipating mechanisms such as the counter current heat exchanger. In addition, water has a very high specific heat, and easily conducts heat. Moreover ETP dolphins are not known to have a thick blubber layer. Thus perhaps, under normal conditions, the real problem of *S. attenuata* and *longirostris* is actually to not be cold. In fact, hyperthermia may never occur in healthy cetaceans kept in water. A possible corollary is that ETP dolphins must be constantly active to generate enough heat to maintain their body core temperature.

Considered together these observations suggest that ETP dolphins are protected from hyperthermia by the medium they inhabit and most likely, also, by highly efficient physiological mechanisms for heat dissipation.

CIE-SO5. Tagging and tracking of Stenella spp. during the 2001 Chase Encirclement stress studies cruise

SJ Chivers, MD Scott

Specific comments

All the apparatuses were kept in place with pins. This reviewer is aware that this is common practice in tracking cetaceans, including beluga. But a wild canid or ungulate suddenly finding itself bearing a very large apparatus (proportionally to its body size) attached to its ear or to its tail would certainly run for a while before calming down. Are the trauma and pain not expected to alter those dolphin's behavior? Has the answer to this question been validated by tracking dolphins and/or other cetaceans with less invasive (but less convenient) devices or perhaps by observing the tagged animals over a long period?

It is noted that dives were rarely more than 5 m deep. Could the dolphins have had access to cold water during these dives? As in Pabst et al, CIE-SO4, there is no mention about a possible disturbance of the thermocline within the net enclosure. Have the water temperatures been documented at various depths within the net enclosure? Is it possible that the huge purse seine apparatus and the rapid and vast ship maneuvers with this large appendage make the thermocline shallower or that they disrupt it?

In addition, this reviewer suggests reanalyzing in details the speed of dolphins during the CHESS study. An average speed was given. This could mask a series of sprints done at a speed much above normal travel speed.

CIE-SO6. Coping behaviors of spotted dolphins during fishing sets.

E Santurtún and F Galindo

General comments

Included in the factors contributing to both stress and EM are fear and subjection to fear stimuli such as noise over prolonged periods. Thus a universal recommendation in handling wildlife is to carry out capture of wild animals with minimum exciting stimuli (Fowler 1995; Kock et al 1987b). The behavior exhibited by the ETP dolphins in the net enclosure seems to reflect the high level of exciting stimuli. This could predispose ETP dolphins to EM.

Specific comments

Chronic stress induces marked behavioral changes in animals such as aggression and antisocial behavior. Chronically stressed animals may stop eating or, on the contrary show hyperphagia. The effects of chronic stress may also be manifested as alterations in sexual behavior such as frequent masturbation and attempts to copulate (Fowler 1995). Yet, the authors observed mating behavior in 62.5 % of the sets but do not comment on these observations in the Discussion section.

The authors put emphasis on the passive behavior as a means for recovering from the extensive physical effort caused by the chase. The possibility that passive behavior reflects a clinical sign of EM should be considered (see GENERAL COMMENTS).

CIE-SO7. Stress in spotted dolphins (Stenella attenuata) associated with Purse-seine Tuna fishing in the Eastern Tropical Pacific

A. Dizon, A. Allen, N. Kellar, S. Southern.

And

CIE-SO8. Molecular signature of physiological stress based on protein expression profiling of skin.

S. Southern, A Allen, N Kellar, A Dizon.

General comments.

The concept of measuring stress responses at the molecular level using a single test at a reasonable cost is sound and attractive. Recently, DNA microarrays have allowed the quantification of the expression of thousands of genes in a single experiment. DNA microarrays are based on the hybridization of nucleic acids, which in turn relies singly on the knowledge of the coding sequences of these genes**.**

The authors chose to measure gene expression at the protein level instead of the RNA level. To justify this choice, they correctly rationalize that the poor quality of dolphin skin RNA would not allow interpretable results. However the quantification of gene expression at the protein level using antibodies is also troubled by several technical problems of which the most important are addressed below.

The authors have developed several original technical aspects that deserve to be further elaborated. However, several hurdles severely impair the interpretation of the results. The authors tried to quantify the fishing effort, and to determine whether it was correlated with the stress response measured by immunohistochemistry in the skin.

Specific comments

Three major factors determine whether and how easily an antigen can be detected by immunohistochemistry: 1) the type of fixation and antibody, 2) the type of detection method, and 3) the local antigen concentration.

Investigators have little control over the third factor. The second factor (the type of detection) used by the authors is standard. Problems may have arisen with the first group of factors, the type of fixation and antibody (Harlow and Lane 1988).

The methods used for sample preservation greatly influence the results of immunohistochemistry (Van Noorden 1986). Luckily, the normal and stressed reference specimens gave consistent results despite the different methods used for their preservation.

Polyclonal sera are composed of the whole repertoire of antibodies found in the animal that was immunized in the first place to generate the serum. For this reason, to avoid high background and interactions between antibodies of different species, polyclonal sera are not used in pools. Note that monoclonal antibodies can be pooled when it is desirable to increase the signal strength from a single given antigen, and as long as the concentration of the monoclonal antibodies is optimized individually (Harlow and Lane 1988). The conditions, in particular the dilution of the primary and secondary antibodies, must be optimized for each antibody that is used. In the present paper, the optimization method is not described. Thus, it is impossible to evaluate this important aspect of the study.

The use of a "cocktail" composed of 40 antibodies is puzzling. The concentration of each antibody is individually optimized, and then this concentration is decreased 40 times for the "cocktail" (The authors do not clearly state that the concentration is 40 times below the optimized concentration). This cannot work. Most likely, because of the 40 times dilution, only several of the 40 antibodies are solely responsible for the "cocktail" positive reactions, those with the highest affinity and/or the antibodies whose corresponding antigen is most abundant and/or concentrated. It would be important to determine how many and which antibodies are responsible for the positive reactions. This would save lots of expensive reagents.

In addition, the use of two antibodies within the same reaction may lead to interactions and potential interference between the two antibodies, altering the final detection signal (Harlow and Lane 1988). Thus, the use of 40 different primary antibodies within the same cocktail, of which many are polyclonal sera (composed of thousands of antibodies with different specificities) along with monoclonal antibodies of different species (mouse and rabbit), almost guarantees such interferences. And then there are likely interactions between two secondary antibodies directed against two different species.

Investigators in biology and veterinary medicine are routinely faced with a wide variety of animal species for which specific antibodies are often unavailable. Hence, the detection of specific antigens becomes a problem when one use immunohistochemistry or other methods based on the availability of specific antibodies. Fortunately, antibodies raised against a given protein in a given species often show "cross reactivity" against the equivalent protein of a different species.

A single amino acid difference may result in dramatic changes in the structure of a given molecule, and thus in the loss of an epitope (the site recognized by a monoclonal antibody). Thus, it is always highly desirable, if not necessary, to validate cross reactivity, even if the amino acid sequence of the antigen is highly conserved. The corollary is that a different, non-related antigen may be recognized. Validation is most often carried out by Western blot using tissues, cell lines or primary cultures known or assumed to contain relatively large amounts of the specific antigen to be detected (Harlow and Lane 1988). Unfortunately there were no attempts to do this here. The author argued that because of the high degree of conservation of SRP among species this is not necessary. This reviewer disagrees.

In CSE-SO8, the Reference specimens are grouped under Normal and Stressed. Problems arise in that samples are most often from different animals from different species, are collected in different body locations, and are often preserved with different preservative methods (Table 1a). In other words, the validity of the control normal samples is not certain. Adequate controls would have been biopsies taken from the same animal from a given species before and after stress. This is lacking. The description of the specimen sets is not in the Material and Method section but is presented instead in the Results and Discussion section (p. 10).

Problems also arise when the specimen sets are analyzed. The SRP profiles of the specimen sets are found to be more complex than the reference specimens, both quantitatively and in terms of pattern. In this reviewer's experience, an important possible explanation for this is that different methods of fixation or processing tissue were used. For instance, exposure of the samples to sun or heat, a blunt scalpel blade, dehydration of samples may have been responsible for the "banded" pattern.

The classification criteria for the SRP profiles are mixed with the results (in the Results section), which makes it hard to understand what the author has exactly done or observed (see "ALTERED" p.7).

The presentation of quantitative values using a naturalistic color scale rather than numbers is significant. This type of presentation of complex data is a sound and interesting concept. As stated by others, "this alternative encoding preserves all the quantitative information, but transmits it to our brains by way of a much higher-bandwidth channel than the "number-reading" channel" (Eisen et al 1998).

This reviewer suggests also considering that skin exposure to ultraviolet light induces the expression of stress proteins in the skin (Li et al 2001). Thus, sun exposure may complicate the results. It may be worthwhile to report and consider the cloud cover, the time of day and the time spent by the animal in the sun (out of the water) before skin sampling. Skin reaction to sun exposure may also explain (or complicate) the differences mentioned in CSE-SO7 pertaining to the sampling position on the animal's body. "In the 54 tagged/bled and the 166 drive-thru animals, the sample came from the dorsal fin, but when recaptured, the samples came from the back". Skin punches collected from the back were predominantly normal. Most likely, the back is less exposed to sun light than the dorsal fin, even in the normal, free-ranging dolphins. Similarly, the skin from the jaws is expected to be less exposed to sun radiation than the skin of the dorsal fin.

CIE-S10. Investigation of the morphology and autonomic innervation of the lymphoid organs in the Pantropical Spotted, Spinner, and Common Dolphins incidentally entangled and drowned in the tuna purse-seine fishery in the eastern tropical Pacific

T Romano, K Abella, D Cowan, B Curry.

General comments

Others have confirmed atrophy of lymphoid tissue, a major histologic feature of stress first observed by Selye. Thus, describing potential changes in the lymphoid tissues of chased dolphins in response to stress is a straightforward and sound proposition even though baseline data are lacking. .

Specific comments

No changes were found in the lymphoid organs. Here as in CIE-S03, the lack of control animals could have hampered the ability to draw any conclusions. However, the authors compared the microscopic anatomy and innervation of ETP dolphins' lymphoid organs to those of other cetaceans that they previously examined. They rightly conclude that the lymphocyte population observed in the lymphoid organs shows normal density and distribution, and that the innervation of lymphoid organs is also similar to that of other cetaceans, with which this reviewer agrees. I have examined around 129 carcasses of beluga macroscopically and/or microscopically along with a dozen of other cetacean species. The lymphocytic population observed in the lymphoid tissue of beluga inhabiting the St Lawrence Estuary (Martineau, unpubl. observations) is markedly depleted compared to that of ETP dolphins. In contrast, the lymphoid tissue of other cetaceans examined by this reviewer is comparable to that of ETP dolphins, judging by the excellent figures of the paper.

CIE-S11. Histopathological assessment of dolphins necropsied onboard vessels in the Eastern Tropical Pacific Tuna fishery

DF Cowan, BE Curry

General comments

The pathological studies on ETP dolphins were two-fold. 1) Over three years, tissues of dolphins killed in the course of the tuna fisheries have been collected by trained technicians, and subsequently examined by Dr. Cowan. 2) The carcasses of dolphins killed during the CHESS program were collected and processed in the same way.

Overall, 56 dolphins were necropsied onboard by technicians, and the collected samples from these dolphins were later examined histopathologically by Dr. Cowan.

The examination of skeletal muscles is an important part of the post mortem examination of human and animals. ETP dolphins are wild mammals ontogenetically related to ungulates, and are placed by the tuna fisheries in the same conditions that trigger severe skeletal muscle lesions in ungulates. Therefore, EM should be strongly suspected, and the target organ of EM is skeletal muscle. Thus, the examination of skeletal muscles is of central importance. Yet the skeletal muscles are not listed in the Material and Method, the Results or the Appendix (necropsy protocol) sections.

Specific comments.

Most of the lesions described in this paper and found in organs other than the heart have been previously reported in free-ranging dolphins, including ETP spotted and spinner dolphins (Cowan and Walker 1979; Turnbull and Cowan 1998).

Parasitism. In ETP dolphins as in other free-ranging mammals, parasites were found to represent a major cause of diseases (and death).

Heart lesions. *Acute myocardial lesions.* Dr Cowan reports hyalinized and wavy fibers, perinuclear vacuolation and contraction band necrosis in the myocardium. In domestic species, the significance of wavy cardiomyocytes is not clear (Robinson and Maxie 1993).

As commented by the authors, contraction bands result experimentally from excessive release of catecholamines. However, these lesions are also often seen in domestic animals dying from other causes, including EM (Turnbull and Cowan 1998; Bartsch 1977). These lesions are also present in normal hearts when they are fixed soon after death (Robinson and Maxie 1993).

One of the authors also previously reported contraction band necrosis in 100% of 52 stranded cetaceans collected between 1991 and 1996 along the Gulf of Mexico, and reported that these lesions were "part of a capture myopathy type syndrome" (Turnbull and Cowan 1986). This reviewer is not so conclusive about the significance of CBN, because CBN can be found in other conditions, but agrees that these lesions are entirely consistent with EM. Again the examination of the skeletal muscles will be necessary to confirm the occurrence of EM in ETP CHESS dolphins.

In animals, myocardial necrosis related to stress can be divided in two groups; those associated with skeletal muscle lesions, and those that are not accompanied by skeletal muscle lesions. It is not clear however whether skeletal muscle lesions have been always sought in studies describing the second group of conditions (see review: Van Vleet and Ferrans 1986). Myocardial necrosis with skeletal muscle lesions occurs in EM and in the porcine stress syndrome. Myocardial necrosis without lesions to the skeletal muscle is the result of experiments where laboratory animals are submitted to fairly aggressive challenges. Examples include immobilized or restrained rats, overcrowded rats and rabbits, electric shocks in rats and squirrel monkeys, exposure of kangaroo rats to cold, exposure of rats to heat, restraint and water immersion of rats, acceleration of pigs, and several others (Van Vleet and Ferrans 1986).

Chronic myocardial lesions: The authors report myocardial small patchy fibrous scars and hypothesize that these may be the chronic consequences of the cardiac acute lesions described above in survivors. This reviewer agrees with the authors' interpretation of these lesions. We have frequently observed patchy fibrous scars in the myocardium of beluga stranded dead on the St Lawrence Estuary shoreline (Cowan 1966). Although beluga are not the object of chase or entrapment, they are the object of an extensive whale watching industry characterized by dense and continuous circulation of boats of all sizes and speeds that could well mimic the ETP tuna-dolphin fishing.

CIE-S12. Potential effects of chase and encirclement by purse-seiners on behavior and energetics of spotted dolphin (S. attenuata) mother-calf pairs in the ETP.

And

CIE-S13. Energetics consequences of chase by tuna purse-seiners for spotted dolphins (S. attenuata) in the Eastern Tropical Pacific Ocean.

EF Edwards.

General comments

The author supports convincingly and mathematically the intuitive and logical hypothesis that chasing a mother-calf pair can disrupt it, in the same way that a cattle stampede is likely to separate mothers from calves. The author also supports that the likelihood of separation is highest for calves within their first year based on behavioral, physiological and physical evidences.

The disruption of the pair would lead to high stress for the calf and/or possible permanent separation from the mother followed by the calf death. For this reviewer, in the Summary and Conclusions section, more emphasis could be placed on the importance of frequent suckling to support the energetic needs of the very young calf.

The mathematical demonstration of disruption of the calf-mother pair was so convincing that a reviewer asked during the La Jolla meeting how *any* intact mother-calf pair could still even exist during the chase considering that, theoretically, all pairs should be disrupted because of the calf inability to physically sustain the elevated speed. The problem was simply solved: Chased animals do not travel at a constant speed but rather by stops and go.

CIE-S14. Evasive behavior of Eastern tropical Pacific Dolphins relative to effort by the tuna purse seine fishery

SL Mesnick, FI Archer, AC Allen, AE Dizon.

And

CIE-S15. Estimation of reproductive and demographic parameters of the eastern spinner dolphin (S. longirostris orientalis) using aerial photogrammetry

K Cramer, WL Perryman

General comments

It is highly challenging to precisely count beluga in the St Lawrence Estuary despite their white color, their relatively large size and relatively restricted habitat. What to say then about counting ETP dolphins with a single helicopter/aircraft in an area that is, based on rough calculations, about 5% of the Earth surface, and considering that ETP dolphins do not harbor a remarkable color at all? The authors have to be commended to have carried out such a gigantic task.

Specific comments

CIE-S14. This is a fascinating paper. Dolphins spread over 5% of the Earth surface seem to have been trained to exhibit a specific behavior dictated by an extensive international industrial endeavor carried out over five decades.

CIE-S15. In beluga whales living in the St Lawrence Estuary, the proportion of juveniles, defined as grey individuals, was found to be the most sensitive indicator to assess population recovery (Béland et al 1988). If the eastern spinner dolphin is recovering, an increasing proportion of calves would be observed over the years. On the contrary, the authors found a significant decline in proportion calves between survey conducted in the early 1990 and that conducted in 2000. Thus, they found no support for a possible recovery of this population.

RECOMMENDATIONS (Detailed)

The hematological (CIE-S02) and necropsy findings (CIE-S11) are consistent with the hypothesis that the ETP dolphins suffer exertional myopathy (EM) caused by strenuous exercise due to the chase. In turn, EM could cause post-release mortality. To demonstrate this hypothesis, this reviewer suggests the following experiments in decreasing order of feasibility and increasing order of cost.

- **To determine baseline biochemical parameters**: To allow the interpretation of any biochemical parameters measured during the CHESS study, particularly with regards to muscle enzymes and HCO_3 levels, it is important to determine the baseline levels of these parameters in normal, resting domesticated or semi-domesticated spotted dolphins. The only way to achieve this goal is to capture animals and maintain them captive until they become acclimated to captivity. Scott reports that one female pantropical spotted dolphin is kept captive at Gulfarium, Florida (in Curry and Edwards 1998). We strongly suggest that the clinical parameters of this animal - and of all other spotted dolphins that have been kept in captivity since- be measured.

St Aubin et al (1996) have estimated the differences between circulating levels of adrenal hormones of wild and semidomesticated dolphins (*Tursiops truncatus*). A "correction factor" could be estimated and used to reinterpret the ACTH levels measured in the present study.

- **To test the statistical relationship between alterations in CK, AST, LDH, blood pH (acidosis), HCO_3, anion gap, lactic acid and K^+ for each animal with high CK values.**

- **Post mortem examination of dolphins dying in seine with an emphasis on the examination of skeletal muscles.** If skeletal muscles have been sampled over the course of the 2001 study, it is imperative that a trained pathologist examines these samples in order to determine whether EM is present.

The hypothesis that ETP dolphins suffer EM predicts that EM affects between 4 and 36% of ETP dolphins (assuming that affected dolphins do not die of EM, and are not rapidly destroyed by predators, which is unlikely). Each dolphin is captured about eight times a year on the average (Perkins and Edwards1999). Thus, theoretically, any given affected dolphin should show muscle damage at various stages of degeneration and necrosis (acute, versus chronic). In fishing areas where fishing is more intense (and where dolphins are likely to be captured more often), the extent, severity and nature (time wise) of the muscle lesions (acute versus chronic) should be higher than in the areas of lower fishing activity.

Wobeser (1994) has discussed in extensive details the number of animals that must be examined in order to detect a disease in a population of free-ranging animals. For instance, with an animal population size over 1,000,000 animals, and a suspected disease prevalence of 0.5%, 600 animals should be examined. A 2%-prevalence requires that 150 animals be examined. A 10%-prevalence requires that 30 animals be examined.

Scott suggested that 2,500 to 3,000 animals a year are killed by the fishery operations (Curry and Edwards 1998). High priority should be given to the exploitation of this huge, and for the moment wasted, amount of material since it may permit to answer the major question raised by the CHESS study (for this reviewer): Do ETP dolphins suffer EM?

Extensive efforts should be invested to make these carcasses available for standard postmortem examination with an emphasis placed on the examination of skeletal muscles: major groups of skeletal muscles should be examined grossly and histopathologically in order to detect muscle damage. Note that skeletal muscles remain reasonably well preserved for a longer time than most organs, which would allow a delay of several hours between death and the sampling of skeletal muscles. (This time frame may vary with water temperature and exposure of carcasses to solar radiation).

Aqueous humour has been used in humans and in domestic animal species to determine biochemical parameters such as glucose, sodium and chloride urea nitrogen and creatinine concentrations (Cantor et al 1989).

Reproductive studies

Reproductive parameters were collected in the course of the IDCPA Necropsy program for the female pantropical spotted and eastern spinner dolphins. The proportion of lactating and pregnant animals was low. Data collected between 1974 and 1992 showed a wide variation depending of the location where sampling occurred and the year during which sampling occurred.

The carcasses should be examined with regards to reproductive success/physiology. In particular, the ovaries should be examined for the presence of lesions such as follicular cysts and the number of physiological structures such as *corpora lutea* and follicles should be counted. Mammary glands should be examined microscopically for the potential presence of mastitis and/or nematodes that would impair nursing (Curry and Edwards 1998; Geraci et al 1978).

Capture-tagging-release-monitoring experiment:

Dolphins severely affected by EM (or any other lesions) following capture are most likely easier prey for predators. Thus it is improbable that these dolphins can be sampled unless they are tagged before release, and then monitored, followed, and if they die, recovered and examined.

Thus this reviewer recommends capture-release experiments, similar to those that have been carried out to characterize the nature and frequency of EM in terrestrial mammals.

This reviewer suggests the following experimental design. Spotted dolphins should be captured in a simulated fishery, equipped with radio transmitter (check), released, monitored and followed to determine mortality rates after release.

To determine whether there is a causal relationship between the chase and dolphin mortality due to EM, a "dose-response" type of experiment may be carried out: Different levels of intensity may be applied to the chase. Harthoorn and van der Walt (1974) observed that metabolic acidosis was most pronounced in animals that were forced to run at high speed over short distances than in animals that were forced to a lower speed over a longer distance. This reviewer expects that the same relationship will be observed in spotted dolphins. This type of experiment would permit to quantify the impact of the chase and would allow drawing guidelines for the chase process.

If electronically tagged and monitored dolphins do die after release, carcasses should be recovered and examined by a trained pathologist. Based on the frequency of EM in wild ungulates (4% - 36%), the number of spotted dolphins that should be electronically tagged and monitored would be anywhere between 30 and 600 (Wobeser 1994).

No doubt this type of experiment is expensive. However, considering the cumulative costs of research cruises and other types of work carried out over the last two decades and the high degree of uncertainty still in the air, capture-tagging-release-monitoring experiments may in fact be a remarkably cheap endeavor offering a very low cost/benefit ratio.

In addition, this reviewer suggests reanalyzing in details the speed of dolphins during the CHESS study. An average speed was given (Chivers and Scott. CIE-SO5). This could mask a series of sprints done at a speed much above normal travel speed.

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APPENDICES

CIE-SO1. Chase encirclement stress studies on dolphins involved in Eastern Tropical Pacific Ocean Purse Seine Operations during 2001. KA Forney, DJ St Aubin, SJ Chivers.

CIE-S02. Hematological and serum chemical constituents in Eastern Spotted Dolphins (*S. attenuata*) following chase and encirclement. D St Aubin.

CIE-S03. Investigation of the effects of repeated chase and Encirclement on the immune system of Dolphins (*S. attenuata and longirostris*) in the Eastern Tropical Pacific.
T Romano, M Keogh, K Danil

CIE-SO4. Measuring temperatures and heat flux from dolphins in the Eastern Tropical Pacific: is thermal stress associated with chase and capture in the ETP-Tuna Purse Seine Fishery ?D. Ann Pabst, WA McLellan, EM Meagher, AJ Westgate.

CIE-SO5. Tagging and tracking of *Stenella* spp. during the 2001 Chase Encirclement stress studies cruise. SJ Chivers, MD Scott

CIE-SO6. Coping behaviors of spotted dolphins during fishing sets. E Santurtún and F Galindo

CIE-SO7. Stress in spotted dolphins (*Stenella attenuata*) associated with Purse-seine Tuna fishing in the Eastern Tropical Pacific. A Dizon, A Allen, N Kellar, S. Southern.

CIE-SO8. Molecular signature of physiological stress based on protein expression profiling of skin. Š. Southern, A Allen, N Kellar, A Dizon.

CIE-S10. Investigation of the morphology and autonomic innervation of the lymphoid organs in the Pantropical Spotted, Spinner, and Common Dolphins incidentally entangled and drowned in the tuna purse-seine fishery in the eastern tropical Pacific. T Romano, K Abella, DF Cowan, B Curry.

CIE-S11. Histopathological assessment of dolphins necropsied onboard vessels in the Eastern Tropical Pacific Tuna fishery. DF Cowan, BE Curry

CIE-S12. Potential effects of chase and encirclement by purse-seiners on behavior and energetics of spotted dolphin (*S. attenuata*) mother-calf pairs in the ETP. EF Edwards

CIE-S13. Energetics consequences of chase by tuna purse-seiners for spotted dolphins (*S. attenuata*) in the Eastern Tropical Pacific Ocean. EF Edwards

CIE-S14. Evasive behavior of Eastern tropical Pacific Dolphins relative to effort by the tuna purse seine fishery. SL Mesnick, FI Archer, AC Allen, AE Dizon.

CIE-S15. Estimation of reproductive and demographic parameters of the eastern spinner dolphin (*S. longirostris orientalis*) using aerial photogrammetry. K Cramer, WL Perryman

STATEMENT OF WORK

Consulting Agreement Between The University of Miami and Dr. Daniel Martineau

Background

The tuna industry has used the association between tuna and dolphins to fish in the eastern tropical Pacific Ocean for over five decades. Three stocks of dolphins were depleted by high historical levels of dolphin mortality in tuna purse-seine nets, with an estimated 4.9 million dolphins killed during the fourteen-year period 1959-1972. After passage of the Marine Mammal Protection Act in 1972 and the increased use of equipment designed to prevent dolphin deaths, mortality decreased gradually during the late 1970s, 1980s and 1990s. While changes in the fishery have greatly reduced the observed mortality of dolphins, there continues to be concern that the fishing methods used are causing stress to the dolphins involved and that such stress may be having a significant adverse impact on population recovery. As a result, the International Dolphin Conservation Program Act (IDCPA) required that research consisting of population abundance surveys and stress studies be conducted by the National Marine Fisheries Service to determine whether the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock". The stress studies mandated in the IDCPA include:

A. A review of relevant stress-related research and a 3-year series of necropsy samples from dolphins obtained by commercial vessels.
B. A one-year review of relevant historical demographic and biological data related to the dolphins and dolphin stocks.
C. An experiment involving the repeated chasing and capturing of dolphins by means of intentional encirclement.

The necropsy program (A) has analyzed samples from about 50 dolphins killed incidentally during fishing operations. Historical biological samples and data (B) have been analyzed at the Southwest Fisheries Science Center (SWFSC) to investigate stress-activated- proteins (SAPs) in the skin in dolphins killed in the fishery and live-sampled via biopsy. Historical data were also examined to assess separation of cows and calves during fishing operations. The Chase Encirclement Stress Studies (C; CHESS) were conducted during a 2-month research cruise aboard the NOAA ship McArthur in the eastern tropical Pacific Ocean from August - October 2001. During this project, the team worked in cooperation with a chartered tuna purse seiner to study potential effects of chase and encirclement on dolphins involved in tuna purse seine operations. Dolphins groups were found to be much more dynamic than previously recognized, making it extremely difficult to recapture groups of dolphins over the course of several days to weeks, as planned. In the end, nine different dolphins were tracked for 1-5 days during the course of the study, including two animals outfitted with a thermal tag that recorded heat flux, temperature, and dive data. Individual radio-tagged dolphins and 1-4 associated roto-tagged dolphins were recaptured on several occasions spanning shorter periods of 1-3 days. Six satellite tags were deployed to record movement and dive data on dolphins that were not recaptured.

Biological data and samples were collected from as many captured dolphins as possible, and include: 70 blood samples, of which 18 were from repeat captures of marked individuals; 283 skin samples, of which 17 were from previously captured and sampled animals; 449 analyzable thermal images; 52 core temperatures; and 95hrs of heat flux data. Females with calves were noted on several recapture occasions, and one known calf was skin sampled during an initial and subsequent capture. All samples and data are being analyzed at SWFSC and other contracted laboratories.

General Topics for Review

This review includes a suite of studies subsumed under the general topic of "Stress Studies". Up to 17 separate papers will be provided covering the studies described below. The general components are as follows:

- Necropsy samples: Analysis of tissues from dolphins incidentally killed in the fishery.

- Blood samples: Analysis of blood samples collected from wild dolphins captured using purse seine methods to assess A) general health, B) immune function, and C) stress response to capture.

- Stress-activated protein studies: Analysis of skin samples to assess levels of stress-activated proteins in dolphins that were A) killed in the fishery B) captured once C) captured repeatedly and D) bow-riding research vessels.

- Thermal studies: Analysis of thermal images, deep core temperatures, and heat flux data derived from thermal tag deployments on wild dolphins.

- Fishery-related behavior: Analysis of behavioral data from dolphins captured using purse seine methods.

- Behavioral ecology: Analysis of tracking data for dolphins captured, tagged, tracked and recaptured during field studies, to investigate school dynamics and movement patterns.

- Cow/calf separation: Analysis of composition of dolphin schools to investigate separation of lactating females and their calves.

- Dolphin swimming energetics: Analysis of the energetic costs of being chased, particularly for lactating females and associated calves.

Documents supplied to reviewers will include draft manuscripts on topics listed above, and a number of background papers (relevant publications and reports).

Specific Reviewer Responsibilities

The reviewer's duties shall not exceed a maximum total of two weeks, including several days to read all relevant documents, three days to attend a meeting with scientists at the NMFS La Jolla Laboratory, in San Diego, California, and several days to produce a written report of the reviewer's comments and recommendations. It is expected that this report shall reflect the reviewer's area of expertise; therefore, no consensus opinion (or report) will be required. Specific tasks and timings are itemized below:

1. Read and become familiar with the relevant documents provided in advance;

2. Discuss relevant documents with scientists at the NMFS La Jolla Laboratory, in San Diego, CA, for 3 days, from 4-6 February, 2002;

3. No later than March 15, 2002, submit a written report of findings, analysis, and conclusions. The report should be addressed to the "UM Independent System for Peer Reviews, " and sent to David Die, UM/RSMAS, 4600 Rickenbacker Causeway, Miami, FL 33149 (or via email to ddie@rsmas.miami.edu).

Signed________________________Date____________